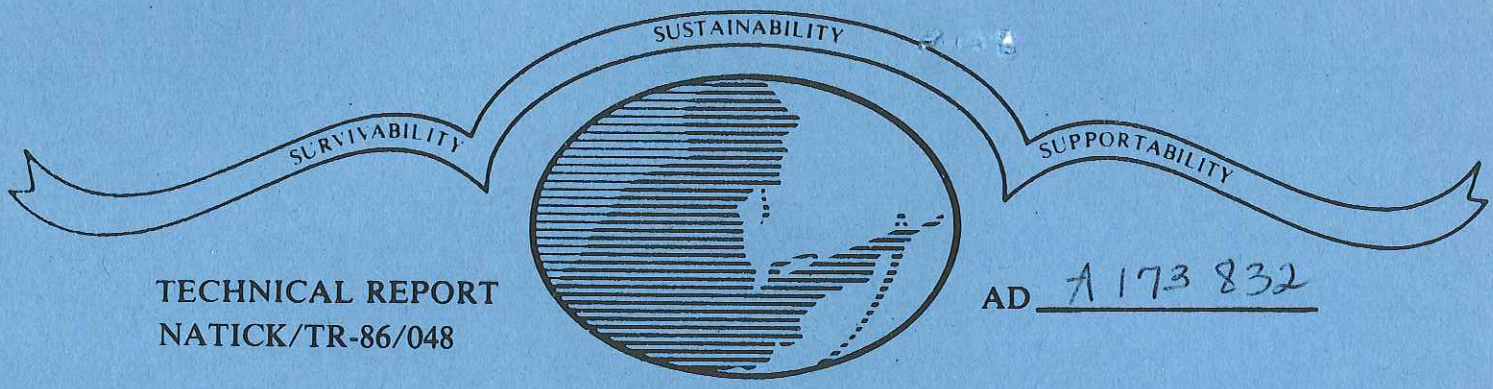

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THE ANTIMICROBIAL EFFECTS OF VARIOUS NUTRIENT ELECTROLYTE BEVERAGES

BY

D.B. ROWLEY
D. JOHNSON AND
G.E. SHATTUCK

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The purpose of this study was to determine if Staphylococcus aureus, Saccharomyces cerevisiae or the mold Aspergillus flavus could survive and multiply in Quenchade, Gatorade, and other electrolyte beverages to the point of creating a stability problem or a health hazard to NBC encapsulated personnel. During periods longer than 6 h, such individuals may need liquid energy and electrolyte supplements (Na+ and Cl-) capable of being consumed through the drinking tube of their protective mask. Although S. aureus was inactivated due the low pH (ca. 3.0) of the elctrolyte beverages, antimicrobial agents sodium benzoate or potassium sorbate had to be added to prevent the multiplication of yeast and mold.

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D. B. ROWLEY

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PREFACE

Various nutrient electrolyte liquids were developed by the Food Engineering Directorate to provide the individual soldier, operating in a Nuclear, Biological, Chemical (NBC) environment while encapsulated in a protective suit, with water, carbohydrate for calories and electrolytes (primarily Na^+ and Cl^-) as specified by an expert committee of the National Academy of Sciences. Such formulations were compared with commercially available electrolyte beverages and their acceptability, microbiological and chemical stabilities were determined initially and over a 24-month storage period at various temperatures.

This study was undertaken to determine if specific bacteria, yeasts and molds could survive and multiply in numerous liquid electrolyte supplements to the point of creating a health hazard to encapsulated personnel operating in a biological or chemically contaminated environment. The work was undertaken during the period January 1981 to September 1982 and was supported by Program 62724A funds.

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INTRODUCTION

In the case of nuclear, biological or chemical (NBC) contamination of the environment, it will be necessary for certain personnel, appropriately dressed and masked, to carry out their mission effectively. The protective mask and clothing may be required for 6 to 24 h or longer and the contaminated environment may not allow normal feeding operations. During periods longer than 6 h, there is a need for a liquid energy and electrolyte supplement capable of being ingested through the drinking tube of the existing M-17A1 protective mask. A liquid supplement should provide the soldier with fluids and electrolytes (e.g., Na^+, Cl^-) lost through perspiration and short- and possibly long-term energy supplied by carbohydrates. Ideally, the supplement should be highly acceptable, microbiologically stable without refrigeration, lightweight and compatible for use with the protective mask drinking tube without causing a microbial hazard.

In 1982, an expert committee of the National Academy of Sciences made various recommendations regarding the short-term (24 h) nutritional support for military personnel in protective clothing.[1] They recommended a beverage be developed to provide individuals with sodium (42 mEq/liter), chloride (38 mEq/liter) and carbohydrates and to supply a minimum of 95 Kcal/liter. The osmolality should not exceed 310 mosmol/liter to ensure palatability and efficient gastric emptying. For such short-term use there is no need for proteins, amino acids, fat, fatty acids or certain

micronutrients (iron, zinc, copper, manganese, trace minerals, or vitamins). Using the above guidelines, the Natick RD&E Center's Food Engineering Directorate (FED) developed a number of different nutrient solutions for masked personnel.

The objective of this study was to determine if certain bacteria (coagulase positive Staphylococcus aureus), molds (Aspergillus flavus), or yeasts (Saccharomyces cerevisiae) were able to survive and multiply in candidate liquid supplements (e.g. Gatorade, Quenchade, a Natick Science and Advanced Technology Directorate (SATD) Behavioral Sciences Division experimental drink, chicken-flavored dental liquid produced by the FED, and the FED-formulated nutrient electrolyte solutions) when incubated at an optimum growth temperature for up to 14 days in both a model system or canteen and drinking tube assembly.

MATERIALS AND METHODS

Test Organisms

Three different microorganisms were used in this study. Saccharomyces cerevisiae was isolated from a commercial package of "active-dry" yeast obtained from a local supermarket. The package was rehydrated according to the directions printed on the package, streaked onto potato dextrose agar (PDA), and incubated at 37°C. From the resultant growth, isolated colonies were picked for streaking on PDA plates which were incubated at 37°C for 48 hours. This step was repeated, and the resultant growth microscopically examined before being streaked on PDA slants for later use. Aspergillus

flavus, aflatoxin negative, was provided by Ms. Bonnie Wiley, Materials Protection and Biotechnology Division, Science and Advanced Technology Directorate (SATD). Coagulase positive Staphylococcus aureus was provided by Mr. Donald Munsey, Biological Sciences Division, SATD.

Media

Potato dextrose agar (PDA), Trypticase soy agar (TSA) and Czapek solution agar (CZA) were used for yeast, staphylococcus and aspergillus cultivation, respectively. All three media were from Difco Laboratories, Detroit, Michigan. Baird-Parker agar fortified with egg yolk-tellurite enrichment (both from Difco Labs) was used when necessary to select staphylococci from other microorganisms.

Nutrient Liquids

Gatorade(R)* (Stokely-Van Camp, Inc., Indianapolis, Indiana) is a popular lemon-lime flavored drink used as a source of energy and electrolytes K^+, Cl^- and Na^+ and has the following constituents: glucose, sucrose, citric acid, sodium chloride, potassium bicarbonate, dehydrated orange juice, and ascorbic acid. It was rehydrated as specified on the package but using distilled water. Quenchade(R)* contains sucrose, glucose, citric acid, salt, potassium bicarbonate, ascorbic acid, artificial flavor, natural

*Gatorade is a registered trade name of Stokely-Van Camp, Inc., Indianapolis, IN, and Quenchade is a registered trade name of Mueller Chemical Co., Inc., Prairie DuSac, WI. Citation of trade names does not constitute official endorsement or approval of the use of such items.

dehydrated lemon juice and artificial colors. Experimental drink (ED), formulated by the Behavioral Sciences Division, SATD, is an unflavored drink that may be used as a source of energy, it contains the following: sucrose, citric acid, and coloring. Chicken and gravy flavored dental liquid is a dehydrated, complete-food source produced by FED. It is rehydrated by adding hot, distilled water and mixing in a Waring blender for 60 seconds. In contrast to the electrolyte beverages, which are true solutions, the dental liquid is a fluid suspension of particulate food materials. None of the above beverages were sterilized before use. In those experiments where indicated, the pH was adjusted by adding either 0.1N HC1 or 0.1N NaOH.

The nutrient electrolyte liquid-sucrose (NLS; pH 2.8 to 3.2) formulated by FED met the recommendations of the National Academy of Sciences and contained sucrose, citric acid, tricalcium phosphate, sodium chloride, xanthan and cellulose gums, orange flavoring and yellow coloring. To NLS either 0.045% potassium sorbate or various concentrations (0.01-0.05%) of sodium benzoate were added as preservatives. Nutrient electrolyte liquids were pasteurized by heating at a product temperature of 77^{o}C for one minute and aseptically poured and sealed in either sterile glass containers or laminated pouches. In a later formulation (nutrient electrolyte liquid-fructose, NLF) by FED the gums were deleted and fructose was substituted for sucrose since it had the greater sweetening power per mole and is not subject to acid hydrolysis leading to increased osmolality during storage.

Inoculum Preparation

S. aureus and S. cerevisiae - Slants of the appropriate media were inoculated and incubated at 37°C for 20-24 h. The growth from each slant was suspended in 5 mL of sterile, distilled water and appropriately diluted to about 10^4 organisms per mL.

A. flavus - Two slants of CZA were inoculated and incubated at 30°C for 72 h. The growth from both slants was suspended in 20 mL of sterile, distilled water, containing 0.1% Tween 80, to give a final concentration of about 10^5 spores per mL.

Cell Survival and/or Growth

S. aureus and S. cerevisiae - Appropriate nutrient liquids (45 or 90 mL) in sterile, cotton-plugged, 250 mL flasks were inoculated with a 10% (v/v) inoculum of the specific organism and incubated at 37° for a period of 24 h to 14 days, depending on the experiment. At indicated time intervals, duplicate 1 mL samples were withdrawn, diluted decimally in chilled, sterile, distilled water, and triplicate 1 mL aliquots pour-plated with the appropriate agar. The plates were incubated at 37°C for 24-48 h, and the six replicate plate counts were averaged.

A. flavus - Mold counts were done as above with the following changes: 1) inoculated flasks were incubated at 30°C for the necessary time; 2) samples were diluted with sterile, distilled water containing 0.1% sterile Tween 80; and 3) pour-plates were incubated at 30°C for 48-72 h. In addition, at each time interval, 5 mL aliquots were centrifuged at 2000 rpm

for 30 minutes in "albumin determination" tubes. These packed cell-mass volumes are referred to in the tables as cell volume, or "C.V."

The canteen and drinking-tube experiments were conducted with the drinking-tube components disassembled from the rest of the protective mask. They were attached to one-quart canteens by a "plug-in" type connector, and the whole assembly placed in a cotton-plugged plastic bag for sterilization. One hundred mL of Quenchade, inoculated with 10 mL of S. cerevisiae were placed in the canteen, incubated at 37°C for 24 h, and sampled at indicated time intervals. Duplicate samples were taken from both the canteen and the drinking-tube; the latter by inverting the canteen, allowing the liquid to flow through the drinking-tube into a sterile container. Cell enumeration was as previously indicated.

Each experiment was repeated at least twice, and the results shown represent the average of all experiments.

RESULTS

Effect of Nutrient Liquids on Viability and Multiplication of Microorganisms

Cells of S. aureus were inactivated when suspended in Gatorade (pH 3.1), Quenchade (pH 2.9) or ED (pH of 3.0) and incubated at 37°C for 24 h (Table 1). However, when suspended under similar conditions in a chicken-flavored dental liquid (pH 6.1), the cells increased from 19×10^3 to 15×10^8 cells/mL (Table 1).

Neither Gatorade, Quenchade nor ED supported active multiplication of yeast cells (Saccharomyces cerevisiae) over a 24 h period at 37°C (Table

(U) TABLE 1. Effect of Various Nutrient Liquids with and Without Potassium Sorbate on Viability of Microorganisms. (U)

Nutrient Liquids	pH	CFU/mL[a] and CV[b]					
		Staphylococcus aureus		Saccharomyces cerevisiae		Aspergillus flavus	
		Initial	24 h	Initial	24 h	Initial	24 h
Gatorade	3.1	35×10^3	1×10^0	68×10^2	101×10^2	18×10^5 CV .005 ml	22×10^5[c] .028 ml
Gatorade + 0.2% potassium sorbate	3.1	ND[d]	ND	21×10^2	2×10^0	17×10^5 CV .005	13×10^4 .009
Quenchade	2.9	31×10^3	0	45×10^2	50×10^0	14×10^5 CV .005	18×10^5 .008
Quenchade + 0.2% potassium sorbate	2.9	ND	ND	42×10^2	12×10^0	9×10^5 CV .005	6×10^5 .005
Experimental Drink (ED)	3.0	12×10^1	2×10^0	54×10^3	23×10^1	13×10^5	12×10^5
ED + 0.2% potassium sorbate	3.0	ND	ND	26×10^3	21×10^0	12×10^5	8×10^5
Dental liquid	6.1	19×10^3	15×10^8	19×10^3	54×10^7	ND	ND
Dental liquid + 0.2% potassium sorbate	6.1	ND	ND	26×10^3	18×10^6	ND	ND

[a] Colony forming units per mL.

[b] CV, measured volume (mL) of cell mass after centrifugation (2000 rpm for 30 min) in "albumin determination" tubes.

[c] Change in cell volume and microscopic and visual examination showed the presence of mycelium. In this case, the viable count is not reliable.

[d] ND - no data

(U) TABLE 2. Effect of Potassium Sorbate in Quenchade and Gatorade on the Viability of *Saccharomyces cerevisiae* and *Aspergillus flavus*[a]. (U)

		S. cerevisiae (cells/mL)			*A. flavus*			
					Initial		14 Days	
Nutrient liquid	pH	Initial	3 Days	10 Days	Cells/mL	Cell volume[b]	Cells/mL	Cell volume
Quenchade	2.9	47×10^1	3	neg.[c]	16×10^5	.005	ND[d]	.035
Quenchade + 0.2% potassium sorbate	2.9	47×10^1	neg.	ND	16×10^5	.005	6×10^1	.005
Gatorade	3.1	ND	ND	ND	16×10^5	.005	ND	.050
Gatorade + 0.2% potassium sorbate	3.1	ND	ND	ND	16×10^5	.005	1×10^1	.005

[a]Nutrient liquids inoculated with *S. cerevisiae* or *A. flavus* were incubated for various time periods at 37 and 30°C, respectively.

[b]Cell volumes (mL) were obtained as in Table 1.

[c]No cells detected when 1 mL of an undiluted sample was plated.

[d]ND, no data.

1). The results indicate that there may have been a 1.5 fold increase when the cells were suspended in Gatorade, and that this apparent increase was prevented by adding an approved food preservative such as potassium sorbate. When suspended in either Quenchade or ED, viable yeast cells declined slowly over a 24 h incubation period. Further incubation in Quenchade showed complete inactivation of the yeast cells (Table 2). The chicken-flavored dental liquid supported active multiplication of the yeast cells, and the addition of potassium sorbate (0.2%) did not markedly reduce this multiplication (Table 1).

Spores of the mold Aspergillus flavus neither developed into actively dividing vegetative cells nor were inactivated when suspended in either Quenchade or ED for a 24 h period, as evidenced by total viable counts. The absence of any major multiplication was also evident from the measured volume of cell mass (Table 1) and microscopic observation (data not shown).

A. flavus spores did develop into multicellular mycelium in Gatorade (Table 1). Although the initial (18×10^5 cells/mL) and 24 h counts (22×10^5 cells/mL) appear to be about the same, the change in cell mass indicated the presence of mycelium. The existence of mycelium was supported by microscopic and visual inspection (data not shown). In the 24-h case, the spores germinated and developed into multicellular mycelia. The latter does not allow accurate cell counts and one must depend on the change in cell mass and microscopic examination to show multiplication. The transition from spore to mycelium in 24 h was prevented by the addition of 0.2% potassium sorbate.

A. flavus multiplied in both Quenchade and Gatorade at 30°C when incubated for 14 days (Table 2). The addition of potassium sorbate to a final concentration of 0.2% prevented the transition from spore to mycelium and resulted in a decrease in viable A. flavus conidia (Table 2). Microscopic examination showed that the fungal conidia germinated but did not multiply in the presence of 0.2% potassium sorbate.

Effect of Nutrient pH on the Inactivation or Multiplication of Microbial Cells.

It appears from the previous studies (Tables 1 and 2), that pH is an important factor in preventing the multiplication of microorganisms in nutrient electrolyte liquids[2]. This was confirmed by the data shown in Figures 1 and 2. Quenchade has a pH of 2.9. At this pH S. aureus was rapidly inactivated (Fig. 1). If the pH were adjusted to pH 4.5, cells of S. aureus were not inactivated as rapidly as at pH 2.9. Yeast cells tolerated pH 2.9 and only a few cells appeared to be inactivated. At pH 4.5 the yeast cells remained viable and in a 24 hour-period increased from 4.2×10^2 to 77.0×10^3 cells/mL.

A pH of 3.1, typical of Gatorade, also inactivated S. aureus cells (Fig. 2) but not as rapidly as the pH 2.9 Quenchade (Fig. 1). If the pH of Gatorade was increased to 4.5, the cells remained viable but didn't multiply during the 24 h incubation period. The effect of pH on yeast cells was similar to that shown in Quenchade.

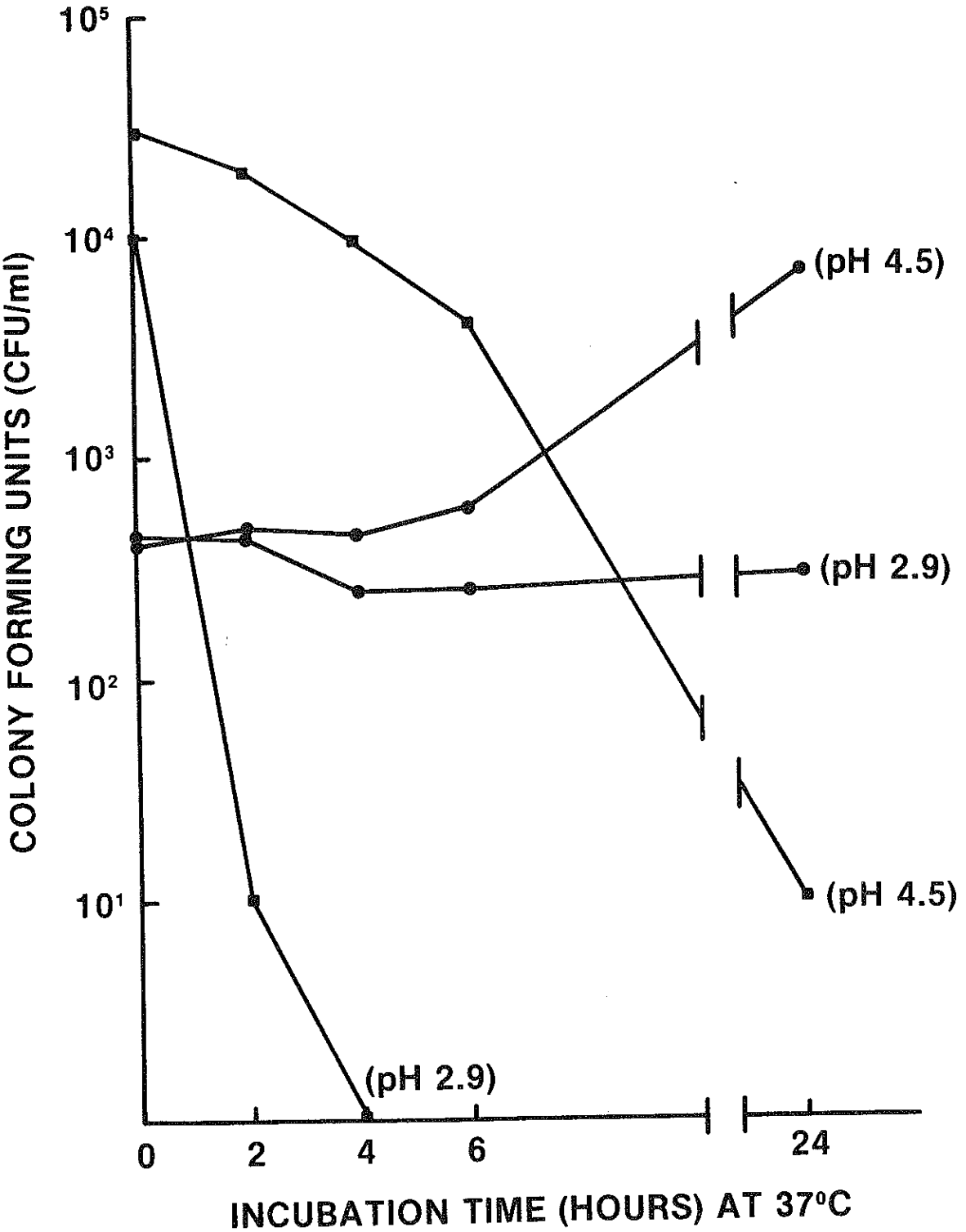

FIGURE 1. EFFECT OF pH OF QUENCHADE ON VIABILITY AND/OR MULTIPLICATION OF *S. AUREUS* AND *S. CEREVISIAE*. SYMBOLS: CIRCLES, *S. CEREVISIAE;* SQUARES, *S. AUREUS*.

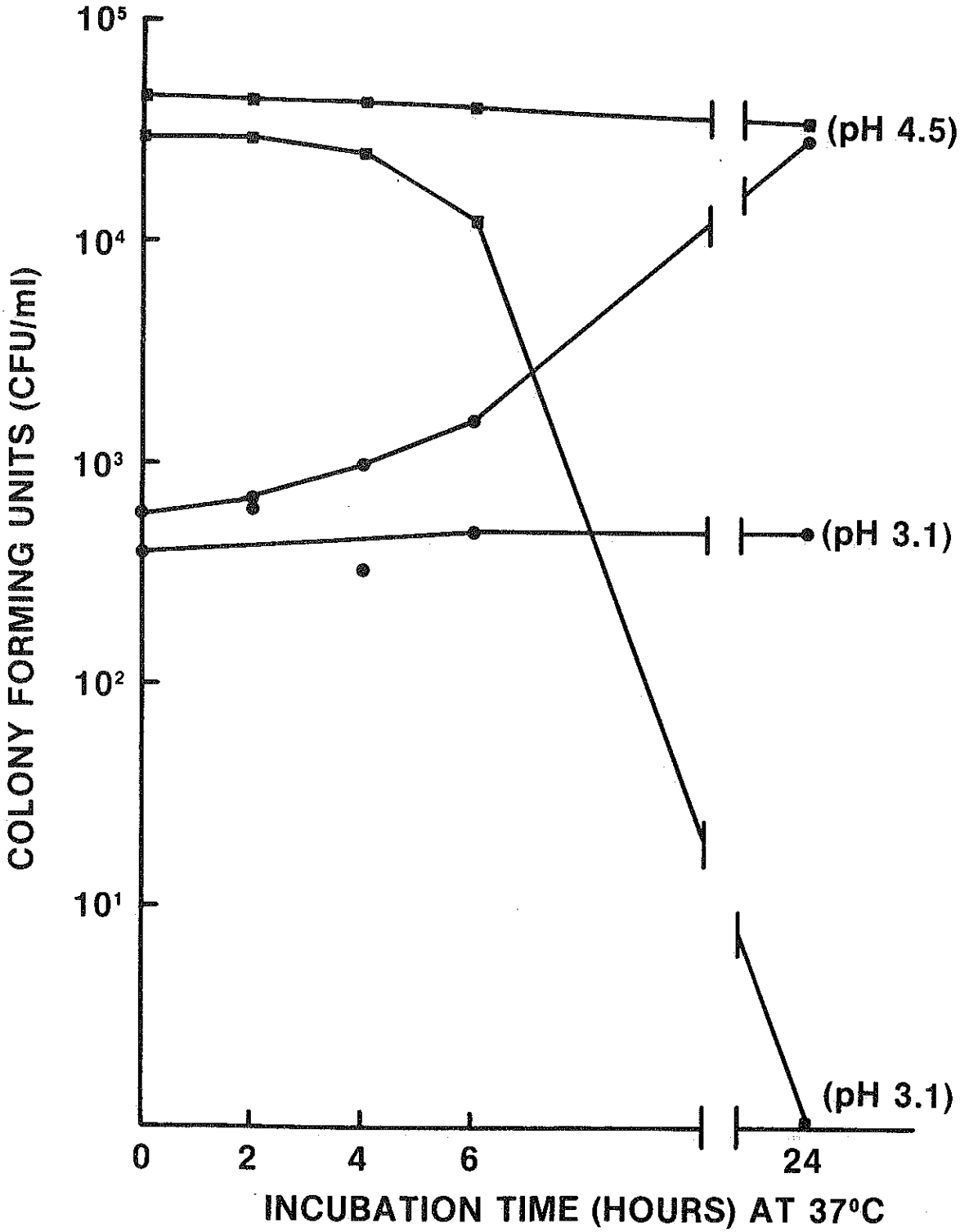

FIGURE 2. EFFECT OF pH OF GATORADE ON VIABILITY AND/OR MULTIPLICATION OF *S. AUREUS* AND *S. CEREVISIAE*. SYMBOLS: SAME AS FOR FIGURE 1.

Inhibition of Fungal Growth in NLS with Potassium Sorbate or Sodium Benzoate

Prior to inoculation the NLS solutions were shown to be free of microbial growth after incubation at 30° or 37°C for 48 h. During two to three days of incubation, neither inoculated A. flavus nor S. cerevisiae, respectively, multiplied in NLS (Table 3). In fact, viable yeast cells decreased from 63×10^4 to 68×10^1 within the first three days. However, as the incubation period was extended beyond 3-4 days, yeast and mold cells showed an increase in numbers. The addition of either 0.05% sodium benzoate or 0.045% potassium sorbate prevented fungal growth during a 14-day incubation period.

Inhibition of Aspergillus flavus in NLF by Sodium Benzoate

Prior to inoculation, these liquids were shown to be free of microbial contamination after incubation at 30°C or 37°C for 48 h. NLF like NLS supported the growth of Aspergillus flavus. After 2 days of incubation, all inoculated samples, containing various concentrations of sodium benzoate, showed some loss of viable conidia, but no viable cells were detected in the sample containing 0.05% sodium benzoate (Table 4). Microscopic examination of this sample indicated that no germination occurred. The addition of 0.05% sodium benzoate prevented outgrowth and allowed very limited germination as judged by microscopic examination. The samples containing 0.01% or 0.015% or 0.02% sodium benzoate showed germination, outgrowth and some increase in cell volume by 5, 5 and 9 days, respectively. All of these three samples and the one with 0.025% sodium benzoate contained visible mycelium by the 14th day.

(U) TABLE 3. The Effect of Potassium Sorbate or Sodium Benzoate in Nutrient Electrolyte Liquid-Sucrose (NLS) on the Viability of *Aspergillus flavus* and *Saccharomyces cerevisiae*[a]. (U)

Organism	Nutrient liquid (NLS) pH 2.9		NLS + 0.045% potassium sorbate pH 3.1		NLS + 0.05% sodium benzoate pH 3.1	
	cells/mL	cell volume[b]	cells/mL	cell volume	cells/mL	cell volume
A. flavus						
Initial	23×10^5	.025	18×10^5	.021	24×10^5	.027
Day 2	ND[c]	.025	8×10^2	.017	1×10^1	.030
Day 14	ND	.061	13×10^0	.013	neg.[d]	.018
S. cerevisiae						
Initial	63×10^4	ND[c]	63×10^4	ND	63×10^4	ND
Day 3	68×10^1	ND	8×10^0	ND	2×10^0	ND
Day 10	56×10^4	ND	neg.	ND	neg.	ND
Day 14	1×10^6	ND	neg.	ND	neg.	ND

[a] A nutrient liquid (sucrose, citric acid, tricalcium-phosphate, sodium chloride, xanthan and cellulose gums, orange flavoring and yellow coloring) inoculated with *A. flavus* or *S. cerevisiae* was incubated for various time periods at 30° and 37°C, respectively

[b] Cell volumes (mL) were obtained as in Table 1.

[c] ND, no data.

[d] No cells detected when 1 mL of an undiluted sample was plated.

(U) TABLE 4. Effect of Sodium Benzoate in Nutrient Electrolyte Liquid-Fructose (NLF) on the Viability of Aspergillus flavus[a]. (U)

Nutrient liquid	pH	Initial	Day 2	Day 5	Day 9	Day 14
NLF + 0.01% sodium benzoate	2.9	cells/mL 2×10^6 CV[b] .006	6×10^5 ND	ND[c] .115	ND .10	ND .10
NLF + .015% sodium benzoate	3.0	cells/mL 2×10^6 CV .006	1×10^4 ND	ND .01	ND .09	ND .10
NLF + 0.02% sodium benzoate	3.1	cells/mL 2×10^6 CV .006	3×10^3 ND	ND .007	ND .012	ND .015
NLF + 0.025% sodium benzoate	3.1	cells/mL 2×10^6 CV .006	1×10^3 ND	1×10^2 ND	4×10^1 ND	4×10^1 .02
NLF + 0.05% sodium benzoate	3.3	cells/mL 2×10^6 CV .006	neg.[d] ND	neg. ND	ND ND	neg. .008

[a] A nutrient liquid (fructose, citric acid, tricalcium-phosphate, sodium chloride, artificial flavoring, yellow coloring, sodium benzoate, and malto-dextrin); inoculated with A. flavus was incubated for various time periods at 30°C.

[b] Cell volumes (mL) were obtained as in Table 1.

[c] ND, no data.

[d] neg., no cells detected when 1 mL of an undiluted sample was plated.

Effect of Quenchade (pH 2.9) on Yeasts in a Canteen with Drinking Tube

Since Quenchade with a pH of 2.9 seemed to be one of the most inhibitory nutrient liquids, it was also tested under simulated use conditions (Table 5) in a canteen with attached drinking tube. Under these conditions (37°C, 24 h) there was a slight decrease in yeast cells.

DISCUSSION

The dental liquid, developed to supply a rather complex mixture of macro and micro nutrients to individuals unable to eat solid foods, was not compatible for any extended use with the current chemical-biological mask. S. aureus, a likely contaminate of a mask and drinking tube under use in field conditions, rapidly multiplied in the dental liquid. Although not determined, it is likely that enterotoxin could also have been produced. Yeasts also multiplied in the dental liquid with or without the addition of 0.2% of the antimicrobial preservative potassium sorbate.

Acidity (pH) plays a major role in the stability of certain foods. Low pH inhibits microbial growth and enhances the effectiveness of certain antimicrobial agents. However, it is not simply a pH effect since propionic and acetic acids are known to be more inhibitory or bactericidal than other food-grade acids at a given pH. Although not well documented, the minimum pH permitting growth of S. aureus is considered to be below 5.0.[2] Yeasts and molds are more tolerant to low pH. In fact, the accepted method for the enumeration of yeasts and molds uses an acidified medium (pH 3.5) to inhibit bacteria.[3] S. aureus was rapidly inactivated when incubated in Quenchade

(U) TABLE 5. Number of Saccharomyces cerevisiae in a Canteen and Chemical Biological Protective Mask Drinking Tube During a 24 h Period at 37°C[a]. (U)

	CFU/mL			
	Initial	4 h	8 h	24 h
Mask drinking tube	75×10^4	71×10^4	66×10^4	57×10^4
Canteen	77×10^4	64×10^4	60×10^4	59×10^4

[a]All experiments used Quenchade as the nutrient liquid.

(pH 2.9), ED (pH 3.0) and Gatorade (pH 3.1) at 37°C. As expected, mold conidia were more stable at low pH. However, except for Gatorade, there was no active multiplication of A. flavus or S. cerevisiae during 24 h. If Gatorade and Quenchade were incubated for 14 days (Table 2), the number of viable yeast and mold cells decreased and increased, respectively. In both Quenchade and Gatorade 0.2% potassium sorbate caused a reduction in the viable cells of A. flavus and S. cerevisiae (Tables 1 and 2).

The NLS developed by FED was microbiologically stable for at least 48 hours. However, further incubation allowed the multiplication of both A. flavus and S. cerevisiae. If sufficient sodium benzoate (0.05%) or potassium sorbate (0.045%) were added, the NLS was microbiologically stable for up to 14 days. Amounts of potassium sorbate below 0.045% or sodium benzoate levels of 0.025% or below allowed measurable amounts of A. flavus growth during the 14 day incubation period as evidenced by increased cell volumes.

Potassium sorbate imparts less of a bitter taste than the benzoates and is frequently used in fruit juices, acid sauces and salads, jams, jellies, soft drinks, fresh and processed cheese and meat products to inhibit the growth of microorganisms.[4] It has a wide spectrum of activity against yeasts, molds and bacteria. Since the pKa of potassium sorbate is 4.8, and it is the undissociated molecule that is responsible for the antimicrobial activity, the sorbate is most effective at pH values lower than 6.0. This fact explains why it had no major antimicrobial effect in dental liquid which has a pH of 6.1 and was very effective in Gatorade (pH 3.1), Quenchade (pH

2.9), as well as the other liquids which all had pH levels around 3.0. Benzoic acid and its salts are also effective against microorganisms when used in foods with a low pH (2.5-4.0). They are frequently used at levels of 0.05 to 0.10%.

When simulating field use by attaching the drinking tube to a canteen and allowing the inoculated Quenchade to flow through the drinking tube about four times over a 24 h period, there was no evidence that yeasts or molds would multiply in either the canteen or drinking tube. Additional safety could be provided by adding 0.045% potassium sorbate or 0.05% sodium benzoate to the Quenchade, or other low-pH liquid in the canteen. Halkiotis, et al. showed that an electrolyte beverage similar to NLS and containing 0.05% sodium benzoate was preferred over the same beverage containing 0.045% potassium sorbate.[5] Therefore, they continued to use sodium benzoate.

Quenchade and Gatorade have a similar pH and supply short-term energy and electrolytes (Na^+, K^+ and Cl^-). FED nutrient electrolyte fluids provide short-term energy and electrolytes (Na^+ and Cl^-). Experimental drink provides only an energy source (sucrose). None of these liquids, especially in the presence of 0.045 to 0.2% potassium sorbate, supports active growth of yeasts, molds or bacteria over a 24-h period at optimum temperatures. Thus, it appears that such nutrient liquids could be consumed while wearing a protective mask in a chemical-biological environment without presenting a microbial health hazard over a 24-h period. If the liquid is to

be used for a period greater than 24 h, it is absolutely necessary that potassium sorbate or sodium benzoate be added.

Rogers et al. showed that various constitutents of nutrient beverages (e.g., flavorants, ascorbic acid, potassium sorbate, dextrose) react with iodine and chlorine and reduce, if not eliminate, their antimicrobial effect.[6] Awareness of this fact is essential in military situations where iodine tablets or chlorine must be used to assure the microbiological safety of field water necessary to rehydrate beverage powders. Rogers et al. indicated that if the iodine tablet was allowed 20 to 30 min in the canteen water prior to adding the beverage powders with halogen demand, the iodine would have fulfilled its antimicrobial effect.[6] However, since there may be little, if any residual halogen the flavored water would not have adequate antimicrobial effect to eliminate subsequent contamination.

Iodine or chlorine would not be required for the pasteurized NBC electrolyte beverage developed by the Food Engineering Directorate that is packed in mask-compatible pouches.[5] Halkiotis et al. showed that NLF had an acceptability equal to or better than commercially available products for electrolyte and liquid replacement.[5] Sensory quality (appearance, odor, flavor, texture) during storage depended on the time and temperature of storage.[7] A consumer acceptance panel rated NLF beverage stored at 4.4°C for 24 months fair to good using the 9-point hedonic scale. At 21.1°C storage the beverage scored lower for flavor and overall quality. When the beverage was stored at 37.7°C, flavor and overall quality were rated unsatisfactory after 9 months of storage. Howker et al. showed that uninoculated samples stored at 4.4°C, 21.1°C and 37.7°C for 12 months

were negative for microbiological growth as judged by counts for aerobics, anaerobics, yeasts and molds, and aerobic and anaerobic thermophiles.[7]

CONCLUSIONS

The common foodborne pathogen S. aureus was very sensitive to low pH levels ($\leq$ 4.5), and therefore did not survive and multiply in electrolyte beverages such as Gatorade, Quenchade, and Experimental Drink. However, S. cerevisiae and A. flavus were more tolerant of such low pH levels. S. cerevisiae was relatively stable, but did not multiply in either Quenchade (pH 2.9) or Gatorade (pH 3.1) over a 24 h period at 37°C. When the pH was increased to 4.5, the yeast cells multiplied within the 24 h period. A. flavus survived and multiplied in both Quenchade (pH 2.9) and Gatorade (pH 3.1) when incubated at 30°C for 24 h.

The multiplication of yeasts and molds was inhibited in all nutrient liquids except dental liquids by either potassium sorbate or sodium benzoate.

Dental liquid (pH 6.1) supported the multiplication of both S. aureus and S. cerevisiae. The addition of 0.2% potassium sorbate did not prevent the multiplication of S. cerevisiae.

The NBC electrolyte beverage containing sucrose (NLS; pH 2.9 to 3.1) supported the multiplication of both S. cerevisiae and A. flavus when incubated at an optimum growth temperature. This multiplication was prevented by the addition of either potassium sorbate or sodium benzoate to a final concentration of 0.045 and 0.05%, respectively.

An NBC electrolyte beverage containing fructose (NLF), instead of sucrose (NLS) was more acceptable to the consumer with added sodium benzoate than with potassium sorbate. Tested concentrations (0.01 to 0.025%) of sodium benzoate below 0.05% did not prevent the multiplication of A. flavus when incubated at 30°C for 14 days.

None of the electrolyte beverages containing an appropriate preservative supported the multiplication of S. aureus, S. cerevisiae or A. flavus. Furthermore, when simulating field use of appropriately formulated electrolyte beverages, there was no evidence that a health hazard would be created in either the canteen or drinking tube of the M-17A1 protective mask.

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